## THE METABOLISM OF β-NAPHTHYLAMINE BY RATS, RABBITS AND MONKEYS

VERY few pure chemical compounds have been reported to cause cancer in human beings. Considerable evidence indicates, however, that β-naphthylamine and benzidine cause cancer of the bladder in industrial workers exposed to these compounds.

This assumption is supported by the fact that cancer of the bladder has been produced experimentally in dogs[1] and in rabbits[2,3] by the oral administration of β-naphthylamine. Other experiments of this type have been unsuccessful, however.[4] Wiley[5] isolated the acid sulfate of 2-amino-1-hydroxy-naphthalene from the urine of dogs fed with β-naphthylamine. The presence of free β-naphthylamine and of a dihydroxy naphthylamine derivative in the urine of dogs similarly treated was suggested by other investigators[6,7] on the basis of color tests.

The metabolic fate of pure β-naphthylamine in rats, rabbits and monkeys has been studied in this laboratory. The compound was dissolved in olive oil and the animals were injected subcutaneously. The urines were collected at an acid pH and chloroform was employed as a preservative. After the addition of sodium hydrosulfite to prevent oxidation, the urine was extracted with ether first at an acid pH, and subsequently at a weakly alkaline reaction. The extracted urine was then hydrolyzed at pH 1 and re-extracted with ether as above. Neutral, phenolic, acidic, basic and phenolic-basic subfractions were obtained from the primary ether extracts.

The ultraviolet absorption spectra of the different fractions were studied with a hydrogen discharge tube as a source of light. With the exception of the fraction containing the acidic compounds, all the extracts of the urine of animals which had been injected with β-naphthylamine showed spectroscopically a series of specific bands. These bands were not given by extracts of the urine of uninjected animals. Identical fractions from the urine of injected rats, rabbits and monkeys showed groups of bands in identical positions. This finding suggests that the three species excrete the same β-naphthylamine metabolites.

From the ethereal subfractions, three substances were isolated by means of high vacuum distillation, chromatographic adsorption analysis and recrystallization. The identities of these three substances were established by mixed melting points, elementary analysis and spectroscopic comparison with synthetic materials. The isolations were controlled by means of spectroscopic methods. The same β-naphthylamine metabolites were isolated in pure form from the urine of animals of the three different species which had been injected with β-naphthylamine, a fact which is in accord with the spectroscopic evidence.

The following compounds were isolated: (1) β-naphthylamine from the basic fraction, (2) N-acetyl-β-naphthylamine from the neutral fraction, (3) N-acetyl-2-amino-6-hydroxy-naphthalene from the phenolic fraction.

TABLE 1

ELEMENTARY ANALYSES[1] OF THE β-NAPHTHYLAMINE METABOLITES ISOLATED FROM URINE

| | | Theoretical Per cent. | Rats Per cent. | Rabbits Per cent. | Monkey Per cent. |
|---|---|---|---|---|---|
| β-naphthylamine | N ..... | 9.79 | 9.77 | 9.75 | 9.82 |
| | C ..... | 83.86 | 83.91 | 83.79 | 83.80 |
| $C_{10}H_9N$ | H ..... | 6.34 | 6.37 | 6.40 | 6.39 |
| | M.P.C.° (uncorr.) | 113° | 113° | 113° | 113° |
| N-acetyl-β-naphthylamine | N ..... | 7.56 | 7.65 | 7.55 | 7.58 |
| | C ..... | 77.80 | 77.77 | 77.72 | 77.77 |
| $C_{12}H_{11}NO$ | H ..... | 5.99 | 6.00 | 6.08 | 6.04 |
| | M.P.C.° (uncorr.) | 132/33° | 132° | 132° | 132° |
| N-acetyl-2-amino-6-hydroxy-naphthalene | N ..... | 6.97 | [2] | 7.04 | 6.92 |
| | C ..... | 71.64 | | 71.65 | 71.60 |
| | H ..... | 5.47 | | 5.54 | 5.56 |
| $C_{12}H_{11}NO_2$ | M.P.C.° (uncorr.) | 223/24° | 223° | 223/24° | 224° |
| Methyl ether of N-acetyl-2-amino-6-hydroxy-naphthalene | N ..... | 6.51 | 6.54 | | |
| | C ..... | 72.52 | 72.54 | | |
| | H ..... | 6.09 | 6.36 | | |
| | O $CH_3$ .. | 14.42 | 14.4 | | |
| $C_{13}H_{13}NO_2$ | | | | | |

[1] Elementary analyses were made by the courtesy of Dr. A. Elek, Rockefeller Institute for Medical Research.
[2] Elementary analysis was made on the methyl ether.

In the fraction containing the phenolic bases from the non-hydrolyzed urine spectroscopic bands were seen in positions identical with those given by 2-amino-6-hydroxy-naphthalene,[8] a fact which suggested the presence of the free compound. After acid hydrolysis of the urine, the same bands could be seen in the phenolic-basic fraction. This suggests that either 2-amino-6-hydroxy-naphthalene or the acetyl derivative, or both, are excreted in part after conjugation as ethereal sulfates or glucuronates, forms which are not ether soluble.

From the urines of rats a very small amount of a naphthylamine derivative was isolated which has not yet been identified. Its properties suggest that it is a

[1] W. C. Hueper, F. H. Wiley and H. D. Wolfe, *Jour. Ind. Hyg. and Toxicol.*, 20: 46, 1938.
[2] S. Perlmann and W. Staehler, *Klin. Woch.*, 11: 1, 1932.
[3] C. Henschen, *Arch. klin. Chir.*, 189: 19, 1937.
[4] I. Berenblum and G. M. Bonser, *Jour. Ind. Hyg. and Toxicol.*, 19: 86, 1937.
[5] F. H. Wiley, *Jour. Biol. Chem.*, 124: 627, 1938.
[6] A. Kuchenbecker, *Zentr. Gewerbehyg. u. Unfallsverhüt*, 8: 69, 1920.
[7] H. Engel, *Zentr. Gewerbehyg. u. Unfallsverhüt*, 8: 81, 1920, and 12: 35, 1924.
[8] Obtained through the courtesy of Drs. W. Calcott and W. J. Balon, of the Jackson Laboratory, E. I. du Pont de Nemours, Wilmington, Delaware.

dihydroxy aminonaphthalene. In the urine of rabbits and monkeys only traces of this compound were present.

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## BACTERIAEMIA IN LAND-LOCKED SALMON (SALMO SEBAGO) IN MAINE

A HIGHLY fatal epizootic occurred among yearling salmon in a hatchery in Maine during July, 1940. The disease appeared to develop very suddenly, but it is possible that a dorsal-fin disorder which arose late in the winter was related to its development.

External lesions associated with the disease consisted of small, shallow, subcutaneous abscesses seldom exceeding a centimeter in diameter and two millimeters in depth. Perforation of even very small abscesses through a central pin-point opening was very common.

The pseudobranch of all sick and dead fish was intensely hemorrhagic and some fish showed hemorrhages in the gills. A few fish developed protrusion of the eyes with an attendant darkening in color.

Internally all fish showed hemorrhages dorsal to the swim-bladder along the post-cardinals. Some had inflammation of the lower intestine and around the anus. Abscesses and hemorrhages were clearly visible in the liver, spleen and kidneys of over half of the fish.

Microscopic examination of the blood, fluid from the orbit and all internal organs showed numerous bacteria typical for bacteriaemia. The pseudobranch was also heavily infected. From the cultures isolated two types of bacteria were found most frequently. In all cases typical strains of *Bacterium salmonicida* were obtained, while from several fish an additional type of bacterium was isolated.

All strains of *B. salmonicida* isolated on different occasions were identical and cross-agglutinated up to the titre of the homologous agglutinating sera.

The second type was motile and did not liquefy gelatine. Most of the strains of this bacterium were also identical in their biological and serological properties among themselves, but differed entirely from *B. salmonicida.*

Further studies are in progress.

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# SCIENTIFIC APPARATUS AND LABORATORY METHODS

## A DESIGN FOR A SATURATED CALOMEL ELECTRODE[1]

THE electrode was designed with the purpose of avoiding disturbance of the interfaces and contamination of the saturated KCl. It is especially suitable for electrometric titrations of acid-base or oxidation-reduction reduction potentials where flushing of the connecting arm is essential. In most of the calomel cells, the flushing is obtained either by passing saturated KCl through the whole body of the cell or by a three-way stopcock in the connecting arm. In the first case, extreme care must be taken not to alter the potential of the cell through disturbance of the active surfaces, changes of temperature or changes in concentration of the solutions. The second method may lead to leaks of KCl in the solution to be measured or to a break in the continuity of the liquid column. An excellent design is that used by Clark in his oxidation-reduction studies. But its clever, though complicated, arrangement of stopcocks makes it difficult to manufacture except by a very expert glass blower, which accordingly raises its price. In the construction of the cell here described, two standard pyrex stopcocks are used: one (A), a straight, two-way 8 mm O.D. with 2 mm bore in the plug; and one (B), a three-way capillary 7 mm O.D. and 1 mm bore. In the latter the plug is changed for a two-way right angle connection.

The cell is entirely made of pyrex glass. Electrical connection is made by means of a thin platinum wire through a heavy pyrex seal. The platinum wire beyond the seal dips into mercury placed in the bottom of the small lower bulb. This lower bulb has a small hole through which a wire is introduced as an electrical lead to the potentiometer (not represented in the diagram). Once this lead wire is in place a permanent connection may be made by fixing it with a plastic cement, which is made also to close the small hole of the bulb. The lower bulb is not completely filled with mercury, *i.e.,* the mercury does not touch the seal. This avoids any contamination of the purified mercury inside the electrode through a possible defect in the seal around the platinum wire.

The cell is filled completely through stopcock A with stopcock B open to the connecting arm. Once the cell is filled stopcock A is permanently closed. Contamination of the solution with stopcock grease may be

[1] From the Department of Physiology, Tufts College Medical School.